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## Self-Reported Stress, Exercise, and the Cortisol Awakening Response in Female Students

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**Abstract**
**Background**: Female undergraduate students often speak to the stress and stressors associated with their university experience. Some stressors can lead to an elevated cortisol awakening response, but the impact of student-life stress on the cortisol awakening response is understudied. **Purpose**: This study observed self-reported stress levels over the academic year to determine if there was an association between student-life stress and the cortisol awakening response for female undergraduate students while controlling for the day of the menstrual cycle. A secondary purpose was to determine if there was a relationship between self-reported exercise levels and the cortisol awakening response in this population. **Methods**: This observational, prospective cohort feasibility study measured self-reported stress (questionnaire) and cortisol awakening responses (salivary samples) on day 21 of each menstrual cycle in 19 female undergraduate students. Measures were taken for up to five consecutive cycles within the 2018-19 academic year. Exercise habits were self-reported by completing the Godin Leisure Time Exercise Questionnaire. **Results**: Stress and the cortisol awakening response displayed no significant relationship ($p = 0.398$) or changed over time ($p = 0.419$) in the academic year; however, a trend toward low/blunted cortisol awakening response was observed. Exercise levels had a significant positive relationship with the cortisol awakening response ($p = 0.002$). **Conclusions**: Self-reported student-life stress was not associated with the salivary cortisol awakening response. However, self-reported exercise levels were significantly associated with the cortisol awakening response in this cohort. Greater self-reported exercise levels were associated with higher, more normal, cortisol awakening responses among female undergraduate students. **Health & Fitness Journal of Canada 2022;15(4):15-24.** https://doi.org/10.14288/hfjc.v15i4.825

Keywords: Cortisol Awakening Response, Stress, Exercise, Post-Secondary Female Students

## Introduction

Female undergraduate students often speak to the stress and stressors associated with their university experience (Graves, Hall, Dias-Karch, Haischer, & Apter, 2021). Cortisol, a chemical secreted by the hypothalamic-pituitary-adrenal (HPA) axis and known for involvement in mobilizing energy during the "fight-or-flight" response, is considered a biomarker of stress (Nater, Skoluda, & Strahler, 2013). It is a glucocorticoid steroid hormone (molecular formula $C_{21}H_{30}O_5$) produced in the cortex of the adrenal glands and released into the blood. For research purposes, salivary measurement can be utilized because the cortisol secretion pattern in saliva mimics concentrations in circulation (Lippi et al., 2009).

Normal cortisol levels follow a diurnal curve, including a peak approximately 30 minutes after waking in the morning, known as the cortisol awakening response. Cortisol fluctuation is sensitive. Thus, slight variations are often observed as individual differences (Clow, Hucklebridge, Stalder, Evans, & Thorn, 2010; Stone, Schwartz, Smyth, Kirschbaum, Cohen, Hellhammer, & Grossman, 2001). For example, acute response to stress elevates cortisol for approximately 45-60 minutes (Clow, Patel, Najafi, Evans, & Hucklebridge, 1997). Many types of stressors have been reported to increase cortisol excretion in humans, such as physical threats, social stress, isolation, and rejection (Gaffey & Wirth, 2014). The anticipation of an upcoming stressor, such as a sporting event/competition, can also induce an increase in cortisol (Van Paridon, Timmis, Nevison, & Bristow, 2017).

More specifically, stressors can lead to an elevated cortisol awakening response (Schulz, Kirschbaum, Prüessner, & Hellhammer, 1998). For example, greater cortisol awakening responses are seen on stressful workdays in civil servants, as opposed to weekends, when there is inherently lower anticipation of daily stress (Kunz-Ebrecht, Kirschbaum, Marmot, & Steptoe, 2004). Chronic fatigue and burnout have conversely been associated with a blunted cortisol awakening response (Chida & Steptoe, 2009; Roberts, Wessely, Chalder, Papadopoulos, & Cleare, 2004). A recent study with elite male rowers measured salivary cortisol prospectively during training and competition. Blunted cortisol awakening response levels were observed during competition in this study when compared to training days, suggesting a maladaptive response or burnout (MacDonald & Wetherell, 2019).

The impact of chronic student-life stress on the cortisol awakening response needs more understanding as previous literature suggested both increased and blunted morning cortisol when different populations were exposed to various stressors (Kunz-Ebrecht et al., 2004; MacDonald & Wetherell, 2019).

The menstrual cycle may further complicate this outcome among female undergraduate students. A recent study by Ozgocer, Ucar, & Yildiz (2022) suggests that the cortisol awakening response remains relatively stable over the menstrual cycle (Ozgocer et al., 2022). However, mixed findings exist in the literature regarding the relationship between cyclical hormonal fluctuations and cortisol (Hamidovic et al., 2020; Liening, Stanton, Saini, & Schultheiss, 2010; McCormick & Teillon, 2001; Nepomnaschy et al., 2011). Therefore, the purpose of this study was to observe self-reported stress levels over the academic year and determine the impact of student-life stress on the cortisol awakening response of female undergraduate students once per menstrual cycle. A secondary exploratory purpose was to determine the relationship between self-reported exercise levels and the cortisol awakening response in female undergraduate students.

## Methods

This study was conducted in the 2018-19 academic year to examine once-monthly cortisol awakening response measures among female undergraduate students. The study was approved by the Conjoint Health Research Ethics Board of Calgary (REB 18-0459) on August 28th, 2018, and conformed to the standards of the Declaration of Helsinki. Participants provided written and verbal consent.

### *Participants*

Nineteen full-time female undergraduate students (mean age 21.4 ± 2.0; range 20-26 years) with an average year of study of 3.6 ± 1.0 (range 2-6) years participated. Recruitment began in September of 2018 and included posters placed throughout the University of Calgary, as well as an online research posting, and word of mouth. All participants self-reported good health and were recreationally active, completing at least 30 minutes of moderate-intensity physical activity per day (i.e., walking, jogging, cycling, etc.). Menstrual cycles for each participant were self-reported and ranged between 25-31 days in length either due to natural cycling ($n$ = 9) or some form of birth control ($n$ = 10), such as oral contraception (birth control pill), inter-uterine device (IUD), or Nuva Ring. The research coordinator measured height in metres and weight in kilograms at the first data collection timepoint, and body mass index (BMI) was calculated for each participant using the metric formula (BMI = weight/height^2).

### *Study Design*

This observational prospective cohort study was designed to investigate self-reported student-life stress and the cortisol awakening response in this population. We used a feasibility design framework to build on previously published studies and acknowledge the participant burden of testing across the menstrual cycle over the 2018-19 academic year (Bowen et al., 2009) with a coordinated sample collection day. Stress and cortisol awakening response levels were collected once per month (October to March), and all samples were obtained on day 21 of the participant's menstrual cycles, based on a previously employed estimated date of the mid-luteal phase (Stanford et al., 2006). This allowed consistent data collection across menstrual cycles without the use of additional invasive hormonal testing measures, thereby limiting participant burden.

Self-reported stress was measured by the Student-Life Stress Inventory (SSI) questionnaire, composed of 51 items in nine categories: frustrations, conflicts, pressures, changes, self-imposed stress, physiological experiences, emotions, behaviours and cognitive appraisal. An overall stress level score was calculated out of a maximum of 255 points (Gadzella, 1994). The SSI questionnaire was developed to "reflect students' life experiences on and off campus" and was based on a theoretical model by Morris regarding types of stressors and reactions to stressors (Gadzella, 1994; Gadzella & Baloglu, 2001;). The SSI and its subcategories have been tested for reliability and validity, with Cronbach's alphas for each category ranging from 0.52 (frustration category) to 0.85 (change category) and an overall questionnaire score of 0.78. Based on Nunnally (1978), a minimum Cronbach's alpha of 0.7 is required to demonstrate internal consistency; therefore, the overall SSI score met this minimum threshold. In a previous study, the questionnaire was repeated twice in a 3-week period, and the results were compared, leading to a Pearson product-moment correlation of 0.78 (Gadzella, 1994). For this feasibility study, participants were instructed to fill out the questionnaire on day 21 of each menstrual cycle throughout the academic year with regard to their experiences over the prior seven days. We previously employed this survey questionnaire to measure student stress (Crack & Doyle-Baker, 2020).

Salivary collection kits (Labrix, Doctor's Data, Inc., St. Charles, IL) were provided for participants to measure their cortisol awakening response (30 minutes after waking, based on the diurnal cortisol profile) on each day 21 of the menstrual cycle. Samples were stored in a standard freezer temperature between -20 to -30 degrees Celsius. The samples were sent to the Doctor's Data Labrix Laboratory for analysis on two occasions, first approximately halfway through the study and second upon completion of data collection. Physical activity levels were self-reported using the Godin Leisure Time Exercise Questionnaire (Godin, 2011).

### *Statistical Analyses Methods*

The Mann-Whitney U test (or the Wilcoxon-Mann-Whitney test) was employed to determine if differences in SSI score and cortisol awakening response levels existed between naturally cycling participants and birth control users. Mixed-effects longitudinal analyses were employed to investigate any changes in SSI scores and the cortisol awakening response over the academic year, and a linear regression was used to determine the relationship, if any, between SSI scores and cortisol awakening response levels.

A post-hoc regression analysis was employed to investigate the relationship between cortisol awakening response levels and self-reported exercise habits. The October data was removed from the analysis due to low sample size and one additional cortisol awakening response sample was removed from the statistical analysis because it was greater than 3 standard deviations above the mean cortisol awakening response value. This may have been an inadvertent participant error in data collection methodology. The participant may have spit slightly into the saliva collection tube, rather than passively drooled throughout the full sample collection, or the participant may not have been able to immediately place the sample in the ice cooler provided, either of which could have compromised this sample (Bhattarai, Kim, & Chae, 2018). All statistical analyses were completed using STATA 17.0 software with an alpha significance level of 0.05.

## Results

The mean height (m), weight (kg) and BMI (kg/m$^2$) of all participants were 1.67 m ± 0.08 (*SD*), 67.83 kg ± 6.69 (*SD*) and 24.40 ± 3.41 (*SD*), respectively. No significant differences were observed between naturally cycling participants ($n$ = 9) and participants using birth control ($n$ = 10) in height ($p$ = 0.167), weight ($p$ = 0.091), BMI ($p$ = 0.214) or year of study ($p$ = 0.794), but birth control users were slightly older than naturally cycling participants ($p$ = 0.026). The Mann-Whitney U test demonstrated no differences in SSI ($p$ = 0.611) or cortisol awakening response ($p$ = 0.805) between naturally cycling participants and those using birth control; therefore, the groups were collapsed and analyzed together. The overall SSI mean score was 135.94 ± 28.34 (*SD*) out of a total possible stress score of 255. The overall mean cortisol awakening response was 11.37 nmol/L ± 4.58 (*SD*), compared to a normal range of 14-25 nmol (Doctor's Data, 2019). Descriptive SSI and cortisol awakening response results for all participants are summarized in Table 1.

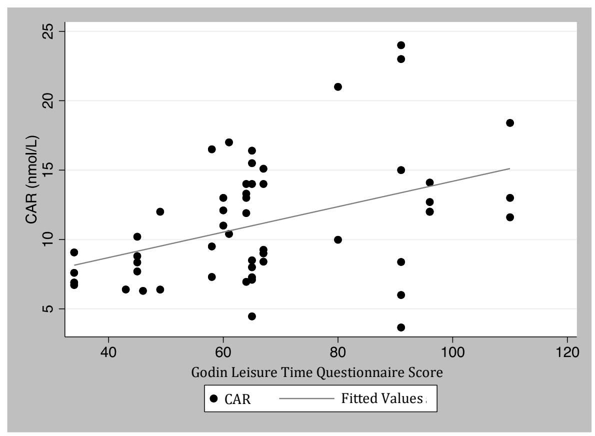
**Table 1. Descriptive results of Student-Life Stress Inventory (SSI) scores and Cortisol Awakening Response (CAR) measures over an academic year (*N* = 19).**

| | SSI (out of 255) | | CAR (nmol/L) | |
|---|---|---|---|---|
| **Time (Month)** | **Mean ± SD** | **Range** | **Mean ± SD** | **Range** |
| **November** | 139.88 ± 22.98 | 107 - 175 | 13.54 ± 6.72 | 6.30 - 24.00 |
| **December** | 124.62 ± 23.97 | 89 - 163 | 11.69 ± 4.46 | 6.40 - 20.00 |
| **January** | 141.82 ± 34.38 | 92 - 200 | 10.00 ± 3.40 | 6.00 - 15.00 |
| **February** | 130.23 ± 33.99 | 76 - 197 | 10.71 ± 3.29 | 6.95 - 18.40 |
| **March** | 141.23 ± 22.45 | 100 - 176 | 11.66 ± 5.20 | 3.66 - 21.00 |

Mixed-effects longitudinal analyses demonstrated no significant change in SSI ($p$ = 0.145) or cortisol awakening response ($p$ = 0.419) over time throughout the academic year, and a linear regression suggested no significant relationship between SSI score and cortisol awakening response ($p$ = 0.398) in this sample, with a regression coefficient of -0.73 ($r^2$ = 0.01).

All participants were recreationally active (self-reported Godin Leisure Time Exercise Questionnaire score greater than 24), with a mean exercise score of 67.43 ± 20.19 (*SD*), and no difference between naturally cycling participants and birth control users ($p$ = 0.269). The post-hoc linear regression analysis suggested a significant relationship between self-reported exercise levels (as measured by the Godin Leisure Time Exercise Questionnaire) and cortisol awakening response measurements ($p$ = 0.002), with a regression coefficient of 1.88 ($r^2$ = 0.17). This relationship can be seen in Figure 1. Conversely, no significant association existed between SSI score and self-reported exercise levels ($p$ = 0.822), based on the regression coefficient of 0.05 ($r^2$ < 0.01).

**Figure 1. Relationship between self-reported exercise habits (via the Leisure Time Exercise Questionnaire score) and Cortisol Awakening Response (CAR) (nmol/L).**




## Discussion

Statistical analyses suggested no significant change in self-reported stress levels (via the SSI) or cortisol awakening response across the academic year and no significant relationship between SSI scores and cortisol awakening response measurements. These results suggest that both subjective and objective stress measurements are relatively stable across the academic year in this sample of female undergraduate students. Also, these results contribute to an already inconsistent body of literature regarding the relationship between university student stress and the cortisol awakening response (O'Flynn, Dinan, & Kelly, 2017). A recent study in 2020 examined student cortisol levels across the academic year via hair samples, and their results suggested that cumulative cortisol was highly associated with event stress, such as events pertaining to personal health, work/school or social rejection, rather than the perception of stress (Stetler & Guinn, 2020). Therefore, it is possible that any changes in stress over the academic year were mainly due to perceived stress rather than event stress, which may be overlooked within the analysis of the overall SSI score and cortisol awakening response measurements. Future research should consider distinguishing the measurements of events and perceived stress among university students using independent and valid tools to detect each stressor type independently over the academic year.

It is also possible that the effect of chronic fatigue or burnout (Grossi et al., 2005) is more impactful on the cortisol awakening response in this sample than the effect of stress. The monthly mean cortisol awakening responses presented in Table 1, and the grand mean cortisol awakening response, suggest that this group of students was consistently trending towards the lowest end of the normal cortisol awakening response range (normal: 14-25nmol), i.e., slightly blunted scores. These blunted cortisol awakening response scores may suggest burnout in our sample of students, given a previously reported association between blunted cortisol awakening responses and burnout in existing literature (Grossi et al., 2005). Future research with a validated burnout assessment questionnaire and more frequent cortisol awakening response measurements may assist with investigating the potential for burnout as a mechanism of blunted cortisol awakening response among female undergraduate students.

The post-hoc regression analysis suggested a significant relationship between self-reported exercise levels via the Godin Leisure Time Exercise Questionnaire and cortisol awakening response measurements. Figure 1 displays the trend that higher levels of self-reported exercise were associated with greater, more normal cortisol awakening response measures. This is consistent with existing literature on healthy older adults undergoing exercise intervention, demonstrating increased cortisol awakening response scores and HPA regulation with exercise (Drogos et al., 2019). However, mixed evidence exists in the current body of literature regarding the relationship between exercise levels and the cortisol awakening response across the lifespan (Anderson & Wideman, 2017), and an examination of this relationship among female undergraduate students does not currently exist for comparison. Given our sample's trend toward low or blunted cortisol awakening responses throughout the academic year, this secondary result indicates that exercise may have a protective effect on cortisol awakening response regulation, resulting in higher, healthier cortisol awakening response levels. Once again, further investigation is required to clarify the relationship in this population and should include objective measurements of exercise, such as wearable technology, rather than relying solely on self-report.

***Limitations***

A limitation of the study is that we did not control for sleep. Some research suggests that sleep quality plays a role in cortisol awakening response; however, currently, mixed results exist among females compared to males (Fekedulegn et al., 2018). Future research should consider this relationship to avoid erroneously attributing blunted cortisol awakening response to burnout when it may result from poor sleep quality.

Notably, novel measurement techniques for cortisol profiling are being established with comparable cortisol levels in sweat and salivary samples (Russell, Koren, Rieder, & Van Uum, 2014). In a recent pilot study investigating cortisol dynamics in human sweat using a wireless system, sweat cortisol levels showed a strong correlation to serum cortisol levels. Compared to the salivary sample collection, the potential benefit of this sweat collection technique is the ability to provide "instantaneous and continuous assessments" of cortisol levels (Torrente-Rodríguez et al., 2020).

We used a well-established and valid at-home kit that allowed participants to take a salivary collection 30 minutes after waking to detect their cortisol awakening response. This collection method relies on the unsupervised diligence of the participants and is limited to one moment in time. We recognize that implementing continuous monitoring of cortisol levels using a non-invasive measurement tool with increased frequency could detect rapid response changes to stress and potentially improve the quality of results in future research.

Our study was designed to test the feasibility of a limited data collection, i.e., one measurement per menstrual cycle (day 21), to reduce participant burden based on challenges associated with recruitment. More frequent data collection during the academic year likely led to a large dropout rate. However, daily cortisol awakening response measurements would provide more robust results because cortisol fluctuation is highly sensitive and individualized (Clow et al., 2010; Grossman, 2001; Stone et al., 2001), and more data points would allow for statistical analysis to incorporate both individual fluctuation and meaningful change over time. A larger sample size would also increase statistical power.

Our results will contribute to guiding future protocol development with possible improvements in methodology. For example, as noted above, we limited our data collection to once per menstrual cycle to limit participant burden. Future research methodology, however, should consider more frequent monitoring given the fluctuating hormone exposure over the course of the menstrual cycle, which may help clarify our results as this field of science progresses.

## Conclusions

Student surveys identify stress as a major part of university life (Karyotaki et al., 2020). Ongoing stress can also lead to the development of more serious mental health issues, including anxiety and depression. Many universities in Canada are aware of this and are trying to ensure a mix of student support available to help students cope with or manage their stress levels. It is, however, important to consider the long-term effects of stress on healthy young females (Pascoe, Hetrick, & Parker, 2020). Results of our study suggest that female undergraduate students may be reaching a state of burnout during their undergraduate years, given the low-to-blunted cortisol awakening response measurements observed (Chida & Steptoe, 2009; Roberts et al., 2004). These findings warrant further research, with larger sample size and more frequent cortisol awakening response measurements, to clarify burnout levels in this population. Future research should examine the amount of perceived stress in female university students compared to event stress. If perceived stress is the larger contributor to overall stress scores, it may be beneficial to provide support services dedicated to educating students on how to positively perceive university life rather than strictly providing support to cope with stressful events. Finally, our secondary analysis suggests that exercise may have a protective effect by increasing cortisol awakening response in this group. However, more research is needed to clarify this relationship. This research should include objective measurements of exercise volume. If confirmed, support services encouraging engagement in exercise may be beneficial for cortisol regulation to combat student stress/burnout.

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## Authors' Qualifications

The authors' qualifications are as follows: Laura E. Crack, BSc, MSc; Constance M. Lebrun, MD, MSc; Patricia K. Doyle-Baker, Dr. PH, PhD, CSEP-CEP.

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